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# RANDOMIZED TRIAL OF TRANSFER FACTOR TREATMENT OF HUMAN WARTS

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## SUMMARY

Dialysed transfer factor, prepared from the leucocytes of a donor whose warts had undergone recent spontaneous regression, was used in the treatment of a child with the Wiskott–Aldrich syndrome. The child then had a spontaneous regression at multiple warty areas. A similar relationship was seen in four otherwise healthy patients in a pilot study. A randomized double-blind study of thirty patients failed to confirm a causal relationship between the transfer factor therapy (equivalent to $2{\cdot}1 \times 10^8$ leucocytes) and wart regressions. The need for randomized trials of transfer factor therapy for diseases with a variable natural history is emphasized.

## INTRODUCTION

'Transfer factor' (TF) is a substance contained in extracts from lysed human leucocytes (Lawrence, 1969). Such extracts have been shown to be capable of transferring cell-mediated immunity from immune donors to non-immune recipients. This transfer of immunity has been demonstrated *in vivo* and *in vitro* with a wide variety of antigens. Attempts have been made to exploit this property in therapy of several human diseases, including tumours, congenital immune deficiencies, and fungal and mycobacterial infections (Levin, Spitler & Fudenberg, 1973). Claims of therapeutic efficacy have been made in uncontrolled studies in these conditions. We report here studies of TF in the therapy of verrucae, benign human tumours which are caused by a member of the Papova virus family, human papilloma virus. The stimulus for these studies was a generalized regression of warts in a patient with the Wiskott–Aldrich syndrome under treatment with transfer factor for this congenital immunodeficiency. The studies reported include the first randomized double-blind study with TF.

## MATERIALS AND METHODS

Dialysed TF was prepared according to the method of Lawrence (1969). Two large pools

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were prepared by weekly leukophoresis of the same donor, one for each phase of the study.

For the pilot study, patients of any age were referred from local physicians for treatment of warts. In the randomized trial, only children and adolescents (over 6 years and under 18 years of age) were studied. The patient groups in both studies included a wide spectrum of disease. This included subjects with only one or two warts who had presented recently to a physician for the first time as well as subjects with a long history of multiple warts, some of which had been treated locally with limited success. No patients with genital or laryngeal warts were included.

On the initial visit a list was prepared of the number of sites involved, and the number and size of warts present at each site. The lesions were photographed. The subject's height and weight were recorded and the body surface area calculated from nomograms. At each subsequent visit, each site was examined independently by three observers. They examined the number and size of the lesions, and rated the status according to the following scale: $-$ = worse; 0 = no change; $+$ = partial remission; $++$ = complete remission (complete disappearance of all warts at a site). At the end of the study, the areas were again photographed. Each observer prepared an *overall* evaluation of the success of the therapy, according to the scale given, taking into account the effect at all sites.

At the initial visit a TF dose corresponding to the lysate of $10^7$ leucocytes was given subcutaneously in the deltoid area. The TF was prepared from a donor whose leucocyte extract had apparently resulted in a regression of warts in a recipient with the Wiskott–Aldrich syndrome, as will be described. As there were (in all cases) no adverse reactions, 1 week later a dose corresponding to $10^8$ leucocytes was given. Doses of this size have been shown capable of converting a delayed skin test to microbial antigens in normal recipients (Lawrence, 1969). The patient was observed weekly for 2 weeks, then an identical ($10^8$) dose given. The patient was then observed weekly for 4 weeks (total study time = 7 weeks). In the randomized trial, the patients were assigned a number keyed to a code held by a laboratory technician. The latter individual sent to the clinic area numbered syringes following the TF schedule given above or identical syringes containing saline placebo.

The data were analysed with the Student's *t*-test.

Informed consent in writing was obtained from all adult subjects, and from the parents of the children studied.

## RESULTS

### *Genesis of the study*

Following a report of a favourable effect of TF on several abnormalities in a patient with Wiskott–Aldrich syndrome (Levin *et al.*, 1970), a congenital immune deficiency, a series of patients with this condition were begun on such treatment at Stanford. One such 2-year-old child, who had, in addition, multiple cutaneous sites of involvement with verrucae, was given TF corresponding to the lysate of $10^8$ leucocytes. Six days following administration of TF, erythema developed at all wart sites. Over the next 1–2 weeks, all warts regressed completely. The leucocyte donor was questioned, and it was discovered that this white male, in his fourth decade, had had spontaneous regression of several warts 6–12 months previously.

*Pilot study.* Six patients, aged 6–24 years, were entered into this phase of the study. Three patients completed the series of three TF injections, each had three or more different body sites with warts. Two patients, aged 7 and 18, had partial regression of all warts after

the second dose of TF, and complete regression of all warts after the third dose. The third patient, aged 6, had complete regression of warts at two of three sites by the end of the 7-week study period. The remaining three patients received only one or two doses of TF and then were lost to follow-up during the study period. One of these patients had a partial regression of some warts and a complete regression of others before being lost to follow-up. Nothing is known of the outcome of treatment in the other two patients.

*Randomized double-blind study.* Because of these results, this phase was restricted to study of children and adolescents. Thirty patients entered into this phase and completed the course of study. Ten had not had treatment for warts, ten had not been treated for warts for the preceding 6 months, and ten had had a variety of ineffective treatments in the past 6 months, including liquid nitrogen to some areas and a variety of topical agents. They could be categorized into mild, moderate and severe disease by the following criteria: mild = less than three body sites involved, less than five total warts, all warts $\leqslant 0{\cdot}5$ cm in size; moderate = three to five sites, five to ten total warts, warts 0·5–1 cm in size; severe = more than five sites, more than ten warts, any wart $> 1$ cm in size. All patients had at least two criteria for one category and were assigned to that category. There were thus three patients with mild disease, eleven with moderate disease, and sixteen with severe disease.

Some dramatic regressions were seen, including complete remissions within the first weeks of study after the first doses. After completion of the study, the code was broken and the data analysed by a statistician. Thirteen patients had received TF, and seventeen placebo. The overall results are shown in Table 1.

TABLE 1. Therapeutic result in transfer factor and placebo-treated groups

| | Number of patients† | |
|---|---|---|
| Observer rating* | Transfer factor | Placebo |
| 0, 0, 0 | 5 | 8 |
| 0, 0, + | 1 | 3 |
| 0, +, + | 1 | 0 |
| +, +, + | 1 | 1 |
| +, +, ++ | 1 | 2 |
| +, ++, ++ | 1 | 1 |
| ++, ++, ++ | 2 | 1 |

* Ranking by three independent observers is shown. The meaning of 0, +, and ++ is given in text. For example 0, 0, + = two observers rated the patients' disease as showing no overall change and the other indicated a partial remission.

† Not shown are two patients who were not seen at the final visit by one observer. One patient given transfer factor was rated +, + by two observers, and one patient given placebo was rated +, ++ by two observers.

As can be seen, there was no difference between transfer factor and placebo treatment results. There were complete and partial regressions in both groups. Moreover, six patients with dramatic partial responses in the first 2 weeks were evenly divided between the two treatment groups. In no patients did new warts appear during the study period.

There was excellent agreement between the three independent observers. Of twenty-eight patients rated by all three, eighteen received identical scores, and in all instances two of three observers were in agreement. Finally, no individual patient was rated by an observer more than one result category higher or lower than the rating by any other observer.

The study groups were also analysed by several different variables: (a) sex (twelve boys, eighteen girls); (b) disease category (mild, moderate, severe); (c) age (above and below 12 years, seventeen and thirteen patients respectively); (d) previous treatment (none, treatment or no treatment for past 6 months); (e) body surface area (above and below 1·48 $m^2$, fifteen patients each category). The ratings of all patients by each observer was also analysed independently. In none of these subgroups was there statistical significance or any indication of a trend towards statistical significance.

## DISCUSSION

Warts are benign tumours caused by DNA viruses of the Papova group. Evidence for an immune response in man to the human strain of the virus include spontaneous regression of warts, the presence of an inflammatory reaction and mononuclear cell infiltrate during spontaneous regression (Tagami *et al.*, 1974), regression of warts in one area of the body when warts in another area are under treatment (Hellier, 1951), a report of immunity to development of warts after repeated wart extract inoculations (Findlay, 1930), skin hypersensitivity responses to wart extracts (Maderna, 1934), regressions after treatment with autogenous vaccine (Powell, Pollard & Jinkins, 1970), regressions after induction of local contact sensitivity (Lewis, 1973), enhancement of infection in immunosuppressed hosts (Spencer, 1969), and studies with animal papova viruses which indicate both a humoral and cell-mediated immune response in the host (Allison, 1969; Lee & Olson, 1969). Antibody to human wart virus has been demonstrated by complement-fixation (Ogilvie, 1970; Pyrhonen & Penttinen, 1972), electron microscopy (Almeida & Goffe, 1965), immunofluorescence (Matthews & Shirodaria, 1973), passive haemagglutination (Ogilvie, 1970), immunodiffusion (Pyrhonen & Penttinen, 1972; Almeida and Goffe, 1965), and precipitation (Ogilvie, 1970) techniques. Regression of warts is generally thought to be mediated by the cellular immune response (Allison, 1969; Rowson & Mahy, 1967).

The greatest difficulty in interpretation of the negative results in this study is the lack of another test of the competence of the TF preparation. Although the same preparations were used to treat patients with the Wiskott–Aldrich syndrome, and confirmed (V. Marinkovich, unpublished results) the beneficial results reported (Levin *et al.*, 1970, 1973), there was in this instance also no specific antigen to assay the preparation, either *in vitro* or *in vivo*, for its effects on lymphocytes. The donor was skin-tested to a variety of antigens, but did not have a positive reaction to any antigen which does not commonly produce positive reactions in the general population. If such reactivity had been found in the donor, it would have allowed this antigen to be used as a marker. Wart antigen could not be used as markers because standardized assays of cell-mediated immunity to human wart virus have not been devised. Wart virus cannot reproducibly be grown in tissue culture, and antigens prepared

directly from human tissues could present problems in *in vitro* assays because of recognition by reacting cells of contaminating human cellular antigens. *In vivo* testing with antigen preparations derived from human materials could present problems because of this contamination and potential biohazards.

A high rate of spontaneous regression of warts could also explain in inability to show a benefit of TF over placebo. In one study the spontaneous regression rate was 67% in 2 years (Massing & Epstein, 1963). Finally, it is possible that regression of warts may be mediated by humoral immunity, possibly through intravascular thrombosis (Matthews & Shirodaria, 1973), and thus be unresponsive to approaches directed at augmenting cell-mediated immunity.

It is possible that patients with definite immune deficiency diseases may be responsive to lower doses of TF than patients whose immunity appears to be generally intact. Larger doses of TF might have produced a different result in this study. Lawrence (1969) has indicated that TF derived from $8{\cdot}5 \times 10^7$ leucocytes is the minimum dose for systemic transfer of cellular immunity; however, larger doses may be needed in therapy than those required only to transfer skin test reactivity.

In any case, this experience should serve as a caution to claims for therapeutic efficacy of TF in uncontrolled situations. Other modalities of treatment of warts also need to be tested in similar controlled settings before they can be accepted as beneficial in this disease.

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